

**Machine translation JP10147534**

- (19) **Publication country** Japan Patent Office (JP)  
(12) **Kind of official gazette** Open patent official report (A)  
(11) **Publication No.** JP,10-147534,A  
(43) **Date of Publication** June 2, Heisei 10 (1998)  
(54) **Title of the Invention** Neoplasm neovascularity inhibitor and a physc constituent  
(51) **International Patent Classification (6th Edition)**

A61K 35/60 ADU  
AAH  
35/84 AED  
45/00

**FI**

A61K 35/60 ADU  
AAH  
35/84 AED A  
45/00

**Request for Examination** Tamotsu**The number of claims** 9**Mode of Application** FD**Number of Pages** 9(21) **Application number** Japanese Patent Application No. 8-324595(22) **Filing date** November 19, Heisei 8 (1996)(71) **Applicant****Identification Number** 593088108**Name** Yakida \*\*\*\***Address** 1-1-21, Osawa, Mitaka-shi, Tokyo(71) **Applicant****Identification Number** 591127917**Name** Seishin Enterprise, Inc.**Address** 5-27-7, Sendagaya, Shibuya-ku, Tokyo(71) **Applicant****Identification Number** 000004341**Name** Nippon Oil & Fats Co., Ltd.**Address** 4-20-3, Ebisu, Shibuya-ku, Tokyo(72) **Inventor(s)****Name** Yakida \*\*\*\***Address** 1-1-21, Osawa, Mitaka-shi, Tokyo(74) **Attorney****Patent Attorney****Name** Yasutomi Yasuo (besides one person)

---

**(57) Abstract**

**Technical problem** the shark as neoplasm neovascularity inhibitor -- the physc constituent which can prescribe a cartilage for the patient effectively as an anticancer agent is offered, still such neoplasm neovascularity inhibitor and IL-12 inductor are used together, and a useful anticancer agent is offered.

**Means for Solution** the inside of a fats-and-oils matrix -- an impalpable powder-like shark -- the neoplasm neovascularity inhibitor which is made to carry out embedding of the cartilage and comes to coat said matrix front face further by other lipids in which the melting point differs from said fats and oils.

**Claim(s)**

**Claim 1** the inside of a fats-and-oils matrix -- an impalpable powder-like shark -- the neoplasm neovascularity inhibitor which is made to carry out embedding of the cartilage and is characterized by coming to coat said matrix front face further by other lipids in which the melting point differs from said fats and oils.

**Claim 2** an impalpable powder-like shark -- the neoplasm neovascularity inhibitor which consists of a cartilage -- it is -- the shark of the shape of said impalpable powder -- the neoplasm neovascularity inhibitor whose cartilage is that to which a thing with a particle size of 32 micrometers or less occupies 99.5 % of the weight or more.

**Claim 3** a shark -- the neoplasm neovascularity inhibitor according to claim 1 or 2 whose cartilage is what uses a great blue shark as a raw material.

**Claim 4** The physic constituent characterized by using neoplasm neovascularity inhibitor according to claim 1, 2, or 3 as a principal component.

**Claim 5** The anticancer agent characterized by using neoplasm neovascularity inhibitor according to claim 1, 2, or 3 as a principal component.

**Claim 6** The cancer treatment approach characterized by sufficient thing to do for amount administration to check neoplasm neovascularity for neoplasm neovascularity inhibitor according to claim 1, 2, or 3.

**Claim 7** The physic constituent characterized by containing neoplasm neovascularity inhibitor and interleukin 12 inducator.

**Claim 8** The anticancer agent characterized by containing neoplasm neovascularity inhibitor and interleukin 12 inducator.

**Claim 9** The cancer treatment approach characterized by using together neoplasm neovascularity inhibitor and interleukin 12 inducator.

**Detailed Description of the Invention****0001**

**Field of the Invention** This invention relates to neoplasm neovascularity inhibitor excellent in the anticancer operation and the analgesic action, the physic constituent containing this, and the cancer treatment approach.

**0002**

**Description of the Prior Art** A shark (shark) is a fish belonging to Chondrichthyes, and inhabits the land part suburbs. the shark which the frame of a shark is a cartilage, and captures and slaughters a shark, and acquires and \*\* the cartilage -- a cartilage has neoplasm depressor effect and that it is effective in cancer treatment gets to know it -- having -- \*\*\*\* -- for example, the product from the U.S. -- a shark -- the car tee RAID which is a cartilage product is marketed as an antitumor agent.

**0003** A neoplasm is 3 1-2mm. If it becomes the magnitude of extent, the neovascularity promoting agent will be produced and it will come to supply own nutrition and oxygen of a neoplasm required for growth. although controlling growth of this neovascularity controls hypertrophy of a tumor cell and various researches on the inhibitory action matter of neovascularity are done from the place effective in cancer treatment -- a shark -- it turns out that a cartilage has the operation which checks this neovascularity.

**0004** however, a shark -- the effective dose for the neovascularity inhibition was very as abundant as per **50-60g** day, the taste and the smell of the cartilage were unpleasant and it was difficult to administer orally in many cases. a shark -- a cartilage is the mixture of a mucopolysaccharide, does not have a medication method suitable besides being based on internal use, and needed to have the property dissolved in the intestines which are absorption parts within the stomach which is strong acid nature at the time of internal use, without dissolving. moreover -- since debility is intense and many of cancer patients are difficult to administer orally in many cases, if such a taste, a smell, and the problem of a dose are not solved -- a shark -- it was in the situation which cannot apply a cartilage as an effective and high anticancer agent of versatility. then, a shark -- utilizing a cartilage as effective neoplasm neovascularity inhibitor was called for strongly.

**0005** By the way, although interleukin 12 (IL-12) is discovered at first as one of the cytokine which has an activity operation of a spontaneous killer cell It becomes clear that it has the multiplication of a T cell (killer T cell) and the activity operation which have specific cell damage

activity to a tumor cell by subsequent research. Further Having the production potentiation of the interferon gamma (IFNgamma) which has the operation to which activation of a killer T cell is urged comes to be admitted, and it is observed by a Homo sapiens cancer patient's therapy as useful matter.

**0006** About IL-12, it succeeded in being produced in large quantities by genetic manipulation in the U.S. recently (recon BINANTO IL-12 (rt-IL -12)). Then, the approach of introducing this interleukin 12 production gene into a cancer cell directly by the transgenics technique is also tried in order to medicate a cancer cell with IL-12 directly.

**0007** In order to utilize IL-12 and to aim at growth loss or disappearance of a neoplasm, the method of making IL-12 of self guide is in the living body besides **which prescribes IL-12 for the patient from the outside** an approach. Such self-IL-12 also had an advantage, like there is no possibility that an unusual immunoreaction may arise, and since susceptibility was high, they were that from which great neoplasm loss quenching is expected.

#### **0008**

**Problem(s) to be Solved by the Invention** the above-mentioned present condition -- taking an example -- this invention -- the shark as neoplasm neovascularity inhibitor -- it aims at offering the physic constituent which can prescribe a cartilage for the patient effectively as an anticancer agent. The purpose of this invention is to use together still such neoplasm neovascularity inhibitor and IL-12 inductor, and also offer a very useful anticancer agent.

#### **0009**

**Means for Solving the Problem** this invention -- the inside of a fats-and-oils matrix -- an impalpable powder-like shark -- It is neoplasm neovascularity inhibitor which is made to carry out embedding of the cartilage and is characterized by said fats and oils coming to coat said matrix front face further by other lipids from which the melting point differs. This invention is explained in full detail below.

**0010** the shark which is the principal component of the neoplasm neovascularity inhibitor of this invention -- a cartilage is indispensable at the shape of impalpable powder. In this specification, the shape of impalpable powder means that the particle size is 1-90 micrometers. the shape of such impalpable powder -- a shark -- in order to obtain a cartilage -- for example, a commercial shark -- it is desirable to impalpable-powder-ize a cartilage. the above-mentioned impalpable-powder-izing -- a shark -- if the protein which is an active principle in a cartilage exceeds 60 degrees C, since thermal denaturation will be carried out and effect will be lost, it is required to carry out to the bottom of low temperature. impalpable-powder-izing under the above-mentioned low temperature -- for example, a shark -- after freezing a cartilage at the moment by -196-degree C liquid nitrogen, coarse grinding can be carried out using cutter type grinders, such as ORIENT Mill, next high-speed rotor mold grinders, such as impeller RUMIRU, can grind with a frozen condition, and it can carry out by applying to air-current type grinders, such as a jet mill, further.

**0011** if in charge of preparation of the neoplasm neovascularity inhibitor of this invention -- the inside of a fats-and-oils matrix -- the shape of above-mentioned impalpable powder -- a shark -- embedding of the cartilage is carried out. as fats and oils which constitute the above-mentioned fats-and-oils matrix, especially if usually used as an object for physic, it will limit -- not having -- for example, animal hardened-oil, **such as vegetable hardened-oil; beef tallow, such as soybean oil rapeseed oil, palm oil, corn oil, and cotton seed oil, lard, and fish oil**; -- it can choose from suitably among such mixture etc. Especially, the fats and oils whose melting point is 42-56 degrees C are desirable. Moreover, anti-oxidants, such as vitamin E and a catechin, may be used together depending on the case.

**0012** the impalpable powder shark of the above -- the shape of the above-mentioned fats and oils in which it is not limited especially as the approach of carrying out embedding of the cartilage, using the above-mentioned fats and oils as a matrix, for example, was made to dissolve, and impalpable powder -- a shark -- embedding can be carried out by making it corn in a high-speed stirring granulator, mixing a cartilage.

**0013** the shape of impalpable powder manufactured as mentioned above -- a shark -- a cartilage embedding fats-and-oils matrix -- next, the above-mentioned fats and oils are other lipids from which the melting point differs, and the front face is coated further. What is necessary is just to choose that in which the melting point differs from the fats and oils used as lipids of this invention in order to produce a fats-and-oils matrix previously, and what has the usually high melting point. Specifically, higher alcohol, such as fatty acids, such as waxes, such as a cull navarho, a rice wax, and yellow bees wax, animals-and-plants hardened oil, stearin acid, and a palmitic acid, natural resin like a shellac, palmityl alcohol, and stearyl alcohol, can

be mentioned. Moreover, in the above-mentioned coating, approaches, such as a powder coating method, can be used, for example.

**0014** if it hits preparing the neoplasm neovascularity inhibitor of this invention -- the inside of others, for example, the fats and oils which carried out melting beforehand, -- the shape of above-mentioned impalpable powder -- a shark -- after distributing a cartilage and carrying out spray cooling, the approach of coating the thing of the shape of impalpable powder of other lipids in which the melting point differs from the above-mentioned fats and oils within tumbling granulator etc. is also employable. **above**

**0015** the neoplasm neovascularity inhibitor of this invention prepared as mentioned above -- a shark -- a cartilage can be used as a principal component and, moreover, the difficulty in internal use can be completely wiped away by masking the taste and a smell completely. Moreover, when friction in the front face of the prepared neoplasm neovascularity inhibitor decreases, the fluidity is improving, adhesion force can decline and it can consider as the very suitable pharmaceutical preparation for internal use. Furthermore, in the strong acid nature situation in the stomach, it does not dissolve but can dissolve in the intestines which are absorption parts.

**0016** the shape of impalpable powder of this invention -- a shark -- as for a cartilage, a thing with a particle size of 32 micrometers or less occupies 99.5 % of the weight or more. It explains in full detail in the example behind -- as -- the shape of impalpable powder of this invention -- a shark -- the particle size of a cartilage has very big effect in the effectiveness, and if particle size is too large, neoplasm neovascularity inhibitory action will fall. a shark -- as the approach of arranging the particle size of a cartilage -- the shape of impalpable powder -- a shark -- the approach of making the sieve of a desired mesh passing a cartilage etc. can be mentioned.

**0017** the shark used for this invention -- the shark acquired **especially** from a great blue shark although the class of shark used as the raw material of a cartilage is not limited -- a cartilage is desirable. the shark acquired from a great blue shark -- a cartilage has many the protein and the mucopolysaccharides which are the contained active principle, especially they are good. In addition, since the car tee RAID from the U.S. is mixed as a raw material and manufactures the shark of varieties, variation is looked at by the product with a lot and it has become a fault that the effectiveness is also low.

**0018** The neoplasm neovascularity inhibitor of this invention not only has neoplasm neovascularity inhibitory action, but it turns out that it has a positive analgesic action. Especially in the case of terminal cancer, usually, a cancer patient is accompanied by the intense pain. Therefore, when the cancer patient who has a pain is medicated with the neoplasm neovascularity inhibitor of this invention as an anticancer agent, the anticancer operation and analgesic action can express the synergistic effect, and can acquire a very effective curative effect.

**0019** When the neoplasm neovascularity inhibitor of this invention examines how the neoplasm neovascularity inhibition effectiveness is discovered, it is suggested that it is what is depended on apoptosis so that it may explain in full detail in the following examples.

**0020** Although the neoplasm neovascularity inhibitor of this invention is very useful as an anticancer agent, since it is utilizable as other physic applications from having the analgesic effect, the physic constituent containing the neoplasm neovascularity inhibitor of this invention is also one of this inventions. Furthermore, the method of performing an anticancer therapy is also included in the range of this invention by prescribing for the patient only the amount which can discover the neoplasm neovascularity inhibition effectiveness for the neoplasm neovascularity inhibitor of this invention.

**0021** The physic constituent of this invention is characterized by containing neoplasm neovascularity inhibitor and interleukin 12 inductor. The above-mentioned physic constituent constitutes the second this invention. Below, the second this invention is explained in full detail.

**0022** In this specification, the word of "interleukin 12 inductor" does not mean only the matter which can only guide interleukin 12 (IL-12) in the living body, you may be IL-12 themselves, may be an IL-12 transgenics cancer cell, and may be rt-IL -12, and in short, especially if IL-12 arise in the living body, it will not be limited. As such a thing, for example, an activation hemicellulose (Active Hemi Cellulose Compound (AHCC)) etc. can be mentioned. As a physiological active substance which carried out enzyme processing of the vegetable fiber contained in the cell wall of the hypha of a mushroom, AHCC is the well-known matter and already contains hetero glucan, such as alpha-(1->4) D-glucan besides beta-(1->3) D-glucan and beta-(1->6) D-glucan, peptide glucan, proteoglucan, lectin, a nucleic acid, an indigestible

nature polysaccharide, etc.

**0023** IL-12 inductor of this invention can mention a mushroom mycelium component besides Above AHCC etc. PSK which is the mycelium component of the shelf fungus which is not limited especially as the above-mentioned mushroom mycelium component, for example, is used as a well-known anticancer agent, SPG which is the mycelium component of Schizophyllum commune Fries, the lentinan which is the mycelium component of shiitake mushroom can be mentioned. Moreover, as such a mushroom mycelium component, mushroom mycelium components, such as AGARISUKU, Ganoderma, NINGYOTAKE, an Agaricus blazei Murill, a NIOU Shimeji mushroom, wall NOR NATAKE, maitake mushrooms, Hericium erinaceum, an oyster mushroom, MANNENTAKE, MUKITAKE, a cob bamboo, KAIGARATAKE, matsutake, an oyster mushroom, a tortoiseshell bamboo, an enoki, and an enoki mushroom, can be mentioned further.

**0024** As IL-12 inductor of this invention, the fungus body component of hemolytic streptococcus etc. can be mentioned further. It is not limited especially as a fungus body component of such hemolytic streptococcus, for example, the well-known anticancer agent of O.K.-432 grade etc. can be mentioned. These are already well-known matter as a biological activator (Biological response modifier (BRM)).

**0025** The IL-12 above-mentioned inductor has the operation to which activation of a killer T cell is urged. Therefore, a killer T cell in the living body is activated with the IL-12 above-mentioned inductor. On the other hand, the neoplasm neovascularity inhibitor explained in full detail upwards controls growth of the neovascularity of a cancer cell (tumor cell). The tumor cell which had growth of neovascularity controlled discovers the adhesion factor of a Fas antigen, CD80, and CD86 grade on a neoplasm front face by the stress by the ischemia. Since the killer T cell has the capacity which carries out antigen recognition of the adhesion factor of such a Fas antigen, CD80, and CD86 grade, these discovered adhesion factor will be in the condition of it being easily recognized by the killer T cell, and getting. And the tumor cell which changed so that it might be recognized easily in this way will become a **killer T cell** is easy to be attacked easily. In such a condition, if the IL-12 above-mentioned inductor exists, as compared with being based on an operation of only IL-12 inductor, it will become easy to attack a tumor cell exceptionally notably. The synergistic effect of the neoplasm neovascularity inhibitor of this invention and IL-12 inductor will be performed according to the above-mentioned mechanism, and a powerful anticancer operation will be discovered with that, and apoptosis will happen, and a tumor cell will be destroyed. This will not be found out without this invention person.

**0026** It is applicable suitably as a pharmaceutical form which it is not limited especially as a gestalt in the case of medicating Homo sapiens or an animal with the physic constituent of this invention, for example, support is made to support, and is used for various drugs. As such support, a solid, a half-solid or liquefied diluent, a bulking agent, and more than an assistant kind for other formulas are preferably used at 0.5 - 90% of a rate 0.1% to 99.5%, for example. Insurance can be medicated with this invention physic constituent taking-orally-wise or parenterally, as a parenteral administration gestalt -- for example, partial administration of in-house administration etc., hypodermic administration, and intramuscular administration -- it \*\* and administers intravenously, and it passes and rectum administration etc. is mentioned. What is necessary is just to prepare the pharmaceutical preparation mold which fitted these medication methods using the technical means of common knowledge common use.

**0027** For example, although it is desirable to set up after taking into consideration a class, extent, etc. of a patient's age, weight, a route of administration, and the illness as for the dose as an anticancer agent, in administration to Homo sapiens, it is usually common to prescribe 1-10g /for the patient in a day in 5-6g /preferably a day in taking orally as an amount of active principles to an adult. Moreover, what is necessary is just to usually prescribe 100-10000mg /for the patient in the 2-5g /day range preferably a day parenterally, although it changes greatly with routes of administration. Depending on the case, less than this is enough and the dosage beyond this may be needed conversely. Moreover, it can divide into 2 - 4 times per day, and a medicine can also be prescribed for the patient.

**0028** The pharmaceutical form of a solid or liquefied dosage unit, for example, an end agent, powder, a granule, a tablet, a capsule, syrups, elixirs or suspension, and others can perform internal use.

**0029** An end agent is manufactured by making an active substance into suitable fineness. Powder is manufactured by mixing with the support for physic which made the active substance suitable fineness and subsequently made it fine similarly, for example, starch, and an edible carbohydrate and other excipients like a mannitol. The thing of a taste correcting agent, a

preservative, a dispersant, a coloring agent, perfume, and others may be blended if needed.

**0030** A capsule is manufactured by being filled up with what granulated an agent, powder, or a tablet after making it the shape of powder as mentioned above first into a capsule envelope like a gelatine capsule. Moreover, lubricant, a plasticizer, for example, the silica of colloid, talc, magnesium stearate, calcium stearate, a solid polyethylene glycol, etc. may be mixed to arbitration before restoration. If disintegrator, a solubilizing agent, for example, a carboxymethyl cellulose, carboxymethyl-cellulose calcium, hydroxypropylcellulose, cross carmellose sodium, carboxy starch sodium, a calcium carbonate, a sodium carbonate, etc. are added, medicinal effectiveness when a capsule is taken in is improvable.

**0031** Moreover, suspension distribution of the impalpable powder of this article is carried out into vegetable oil, a polyethylene glycol, a glycerol, and a surface active agent, and this can be wrapped in a gelatin sheet and it can consider as an elastic capsule.

**0032** A granule adds and kneads binders (for example, carboxymethylcellulose sodium, hydroxypropylcellulose, methyl cellulose, the hydroxypropyl methylcellulose, gelatin, a polyvinyl pyrrolidone, polyvinyl alcohol, etc.) and wetting agents (for example, syrup, starch paste, gum arabic, a cellulose solution, or a high polymer solution etc.) if needed to what mixed an above-mentioned excipient and disintegrator with the active substance made into the shape of powder, subsequently, can carry out forcible passage and can prepare a screen. Thus, after applying to a tableting machine first instead of granulating powder, the slag of the imperfect gestalt acquired can be crushed and it can also be made granulation. Dissolution delay-ized agents (for example, paraffin, a wax, hydrogenated castor oil, etc.), resorption agents (for example, the fourth class salt etc.), or adsorbents (for example, a bentonite, a kaolin, phosphoric acid JIKARUSHIUMU, etc.) may be mixed beforehand.

**0033** A tablet can be prepared by adding and tableting stearin acid, a stearate, talc, and a mineral oil and others as lubricant to the granule made by doing in this way. In this way, film coating and glycolalx may be further given to the manufactured uncoated tablet.

**0034** Without passing through the process of granulation or slugging as mentioned above, after mixing with a fluid inert carrier, you may tablet the active ingredient of this invention directly. The transparency which consists of a sealing coat of a shellac or translucent protective covering, covering of sugar or polymeric materials, covering on \*\* that consists of a wax can be used.

**0035** The constant rate can make other internal use pharmaceutical forms, for example, syrups, elixirs, suspension, etc. dosage unit form voice so that the constant rate of a drug may be contained. Syrups dissolve an active substance in a suitable flavor water solution, and are manufactured, and elixirs are manufactured by using nontoxic alcoholic support. Suspension is prescribed by distributing an active substance in nontoxic support. A suspending agent, an emulsifier (for example, the isostearyl alcohol and polyoxyethylene sorbitol ester which were ethoxylated), a preservative, a taste correcting agent (for example, a PEPAMINTO oil, saccharin), and others can also be added to arbitration.

**0036** You may microencapsulate the dosage unit formula for internal use if needed. This formula can also bring about extension and self-sustaining emission of reaction time by covering again or embedding an active substance into a macromolecule, a wax, etc.

**0037** Hypodermically, intramuscular, or \*\* and intravenous administration can be performed by considering as the injections of liquefied dosage unit form voice, for example, the gestalt of a solution or suspension. These things dissolve or suspend the constant rate of an active substance in the nontoxic liquefied support which suits the purpose of injection, for example, aqueosity and an oily solvent, and are manufactured by subsequently sterilizing this solution or suspension. Moreover, for a vial, after that, a vial and its contents may be sterilized and the constant rate of powder or the freeze-dried active substance may be sealed. In this case, in order to dissolve or mix just before administration, a preliminary vial and support may be prepared. In order to make a parenteral solution into an isotonicity, a nontoxic salt and salting in liquid may be added, and a stabilizing agent, a preservative, a suspending agent, an emulsifier, etc. can also be further used together.

**0038** It can pass and a rectum administration pharmaceutical form can be prepared by kneading an active substance into the suppository base of hydrophobicity or a hydrophilic property, for example, a polyethylene glycol, cacao butter, high-class ester (for example, palmitic-acid millimeter still ester), and those mixture.

### **0039**

**Example** Although the example of manufacture and example of this invention are hung up over below and this invention is explained to it in more detail, this inventions are not these things

limited to seeing.

example 1 of manufacture a shark -- the shape of fines whose mean particle diameter is 27 micrometers at the manufacture high-speed stirring granulator (high-speed mixer: Fukae industrial company make) of the fats-and-oils matrix covering pharmaceutical preparation of a cartilage -- a shark -- the fused beef tallow hardened-oil (melting point of 48 degrees C) 10 weight section was sprayed, having taught the cartilage 60 weight section, having kept temperature inside the plane at 10 degrees C, and applying stirring of 20 - 100rpm. melting fats and oils -- the shape of fines -- a shark, although the matrix was formed incorporating a cartilage this phase -- a front face -- a shark, since the cartilage is exposed and sufficient covering engine performance is not obtained Furthermore, the fines-like rapeseed hardened-oil (melting point of 68 degrees C) 30 weight section with a mean particle diameter of 5 micrometers is stirred for 30 minutes by about 1000 agitating speed rpm in the same agitator granulation inside of a plane as lipids from which the melting point differs on the front face of this primary matrix. The good fats-and-oils matrix covering pharmaceutical preparation which has acid resistance and sustained-release was prepared coordination and by carrying out spreading.

**0040** Example 1 Neoplasm neovascularity depressant action trial by the mouse (shark evaluation of cartilage particle size)  
a DOSARU air sac (dorsal air sac) -- it examined by law. A Millipore filter is stuck on both sides of the plastics ring of 5mm of diameters, and it is 1x106 in it. MH-134 tumor cell of an individual was poured in with culture medium, and the chamber was created. The mouse was divided into one groups **six** and this chamber was inserted in hypodermically **mouse regions-of-back** . the shark shown in the mouse of four groups for four days at the following -- 1000 mg/kg internal use of the cartilage was carried out, and inhibition extent of neovascularity was evaluated on the 5th. another group -- as control -- a shark -- it was made to pass without prescribing a cartilage for the patient at all, and inhibition extent was evaluated similarly on the 5th. Whenever the neovascularity promoting agent from a neoplasm penetrates a Millipore filter, neovascularity carries out hyperplasia to the field which touched the chamber, and is observed in it. The neovascularity of this neoplasm is presenting the shape of RASEN. Inhibition extent of the hyperplasia of this neovascularity was evaluated in accordance with the following valuation bases.

(++) three point: -- (+) two whose hyperplasia of neovascularity is Tsuguaki point: -- (\*\*) 1 in which neovascularity is carrying out hyperplasia point: -- (-) zero in which neovascularity is carrying out hyperplasia a little point: -- It expressed with the percentage reduction as **opposed to / again / a control group in the rate of control (%)** of a score in which the hyperplasia of neovascularity is not accepted at all.

**0041** a shark -- cartilage (1): -- a shark -- as the raw material of a cartilage -- the product from the U.S. -- a shark -- using the car tee RAID which is a cartilage product, this thing was frozen at the moment by liquid nitrogen (-196 degrees C), and coarse grinding was carried out with the cutter type grinder (ORIENT Mill). Next, with the frozen condition, the high-speed rotor mold grinder (impeller RUMIRU) ground, and finally, with the air-current type grinder (jet mill), it ground further, acquired and adopted.

a shark -- cartilage (2): -- the above-mentioned shark -- what did not pass having applied the cartilage (1) to the 32-micrometer sieve was adopted.

a shark -- cartilage (3): -- the above-mentioned shark -- what passed having applied the cartilage (1) to the 32-micrometer sieve was adopted. The result was shown in Table 1.

#### **0042**

**Table 1**

☒ ID=000002

**0043** the shark from the above result -- a shark with the particle size of extent which passes a 32-micrometer sieve also among cartilages -- it turned out that cartilage grinding powder has



effect trial better SHAKU by the mouse. The ICR mouse (a male, five-week \*\*) was divided into five groups by one groups **ten**, each group was medicated with each tested specimen as follows, intraperitoneal was medicated with the acetic acid 2% after progress on the 40th, and the count which the mouse turned round and caused reflection during the 10th - the 20th **after acetic-acid administration** was measured. The tested specimen continued administration until measurement finished after **administration** every day. For 1000 mg/kg internal use and a better SHAKU administration group, the Indacin administration group is a **car tee RAID administration group / 1000 mg/kg internal use and a better SHAKU administration group** Indacin (non-steroid system painkiller.) per day about better SHAKU per day in car tee RAID. It bred without 25 mg/kg internal use and a chondroitin sulfate administration group prescribing a commercial item for the patient and 333 mg/kg internal use and a control group prescribing any chondroitin sulfate (commercial item) for the patient per day per day. The rate of control was expressed with the count percentage reduction (%) to a control group. The result was shown in Table 4. The predominance of better SHAKU was shown also in the analgesic effect.

**0051**

**Table 4**

x	ID=000005

**0052** Example 5 The manifestation Homo sapiens (a male, 80 years old) of the immunological unique antigen by the clinical example of better SHAKU pharmaceutical preparation administration appealed against epigastric region displeasure, and was diagnosed as there being gastric cancer (seal cell cancer) of an Iic advance mold at least in the gastric angle upper part with gastroscopy. The biopsy specimen was used for the electron microscope and the organization biopsy (immunological examination). Since there was myocardial infarction, it was inoperableness. the shark used in the example 1 -- it prescribed a cartilage (3) and (it having been hereafter called "beta SHAKU" in an example 5) for the patient 120g oral every day to the above-mentioned patient. **per day** The electron microscope view and the immunological special staining procedure view (gastroscopy of the 1st time) of the view which shows apoptosis only with the general view of the usual cancer cell before better SHAKU administration are very slight, the adhesion factor (fluorescent antibody technique) of a Fas antigen (special staining procedure) was not accepted, either, and the manifestation of HSP60 (stress protein, heat shock protein fluorescent antibody technique) was not accepted, either. In the gastroscopy after half **two months and** -passing since the 1st endoscope, the circumference of cancer is the view which is apparently covered on normal membrane, and upheaval of surrounding cancer was also becoming flat. To a change special to a cell membrane and cytoplasm not being accepted, vena-contracta fission of the nucleus of a cancer cell becomes clear, and the electron microscope view in the biopsy of the 2nd time is apoptotic. body was accepted. That is, the electropositive view which shows apoptosis in a cancer cell was accepted.

**0053** The immunological special staining procedure was performed in the body tissue of this stage. anti-HSP60 antibody, anti-CD80 antibody, and anti-CD -- the cancer cell was further examined 86 antibody using the anti-Fas antibody. The Fas antigen became a positivity by better SHAKU pharmaceutical preparation administration, and HSP60 and the adhesion factor of CD80 and CD86 became a positivity further. The cancer of the gastric angle upper part had disappeared completely with the gastroscopy of the 3rd time (from the 1st time to about five months after). In the biopsy of a part with gastric cancer, the cancer cell was all negative.

**0054** When the above view was summarized, the Fas antigen which is not accepted before better SHAKU pharmaceutical preparation administration, HSP60 antigen, and the adhesion factor of CD80 and CD86 all became a positivity by after **better SHAKU pharmaceutical**

**preparation administration** 2 months, and half. Then, cancer had disappeared completely with the gastroscopy of the 3rd time. Since the blood flow of a neoplasm was checked by better SHAKU pharmaceutical preparation administration, stress protein (HSP60) was discovered, it was thought that the Fas antigen and the adhesion factor of CD80 and CD86 became a positivity, and, as for this phenomenon, it was proved that a neoplasm is carrying out perfect \*\*\*\* with the endoscope of the 3rd time after that.

**0055** Research of tumor immunology showed that there were two kinds, necrosis (\*\*\*\*) and apoptosis, at the regression of a neoplasm. the phenomenon in which, as for necrosis, nonspecific immunity acts and a neoplasm disappears by factors, such as inflammation, -- it is - a cell membrane and cytoplasm -- the occasion -- a tumor cell is destroyed in order of nuclear denaturation. In this necrosis, histologically, after neutrophil leucocyte, a macrophage, line fibrocyte, etc. permeate, mineralization and fibrosis take place and cancer disappears. Consequently, a trace remains in a part.

**0056** On the other hand, when a neoplasm disappears by apoptosis, inflammation has completely placed and changed to normal tissue, without accepting a trace, without happening. In this case, a killer T cell recognizes the Fas antigen of the front face of a tumor cell, and pastes up, and it turns out that apoptosis is guided to coincidence by the synergy of the adhesion factor of CD80 and/or CD86 grade. After nuclear denaturation (nuclear fission and a nuclear nucleolus (apoptotic body)) takes place first, cytoplasm and a cell membrane are \*\*\*\* (ed) by the macrophage. Therefore, a trace does not remain. Since the inflammation residual views on mineralization fibrosis etc. were accepted in the disappearance part of cancer with neither of the cases, as for the example of better SHAKU pharmaceutical preparation administration, disappearance of the cancer by apoptosis was suggested. Moreover, it was clear that cancer has disappeared extremely for a short period of time (one month - 1 month, a half).

**0057** Example 6 Immunological unique sex-test Sarcoma180 tumor cell by the mouse was transplanted back **mouse** , and contrast with the control group which nothing has prescribed for the patient about the group which injected intraperitoneally TNP470 (Takeda Chemical Industries, Ltd. make) 10mg, the group which administered better SHAKU 1000 mg/kg orally, and the group which did 24microg intraperitoneal injection of Angie Oster Ching (Institute of Chemical and Serum Treatment make) examined the growth depressor effect of a neoplasm. It turned out that any group has controlled tumor growth with a significant difference ( $P < 0.05$ ) as compared with a control group in the 2nd week and the 3rd week. In the neoplasm tissue of this stage, HSP60, the Fas antigen, and the manifestation of CD80 and CD86 were considered on the immunity histology target. Consequently, HSP60, the Fas antigen, and the manifestation of CD80 and CD86 were increasing intentionally.

**0058** On the other hand, when the same experiment as the above was conducted using the nude mouse which is a T cell deficit mouse, although a certain amount of tumor growth depressor effect was accepted, there was no significant difference. In immunity histological examination of neoplasm tissue, HSP60, the Fas antigen, and the manifestation of CD80 and CD86 were positivities. However, the view of apoptosis was not accepted.

**0059** From the above result, the antitumor action of a neovascularity inhibitor is not based only on the ischemia over a mere neoplasm, the killer T cell has recognized change of a qualitative quantitative manifestation of the manifestation of the stress protein in the ischemia condition of a neoplasm or a Fas antigen, CD80, and CD86 grade, antitumor action was demonstrated, and it was thought that apoptosis was guided.

**0060** Examples 7-10 About the cancer disease Homo sapiens who shows in the example table 5 of concomitant use administration of AHCC and beta SHAKU, while prescribing AHCC for the patient every day by the dosage of 3.0g/day, beta SHAKU was prescribed for the patient every day by internal use by the dosage of 20g/day. After prescribing a medicine for the patient for three months, a therapy judging, NK activity, IL-12 numeric value, and the value of CD4/CD8 were measured. In the example 7 and the example 10, each accepted contraction of 50% or more of neoplasm. However, each NK activity is normal values and it turned out that activity is not accelerating. Also in any of examples 7-10, IL-duodecimal showed the high value far across the normal range. In contraction of a neoplasm, it has guessed that IL-12 were involving. In addition, CD4 expresses a helper T cell among Table 5, and CD8 expresses a killer T cell. The value of CD4/CD8 expresses the degree of increase in quantity of a killer T cell, and with **value one or less** , it means that the killer T cell is increasing. Normal values are 1.0-1.5.

**0061**  
**Table 5**

 ID=000006**0062**

**Effect of the Invention** the neoplasm neovascularity inhibitor of this invention -- a shark -- since the taste with an unpleasant cartilage and a smell are vanished, it is acid resistance and enteric, convenience is in internal use and it combines and has an anticancer operation positive moreover and an analgesic action positive moreover, it is very useful as a physic constituent. Since the concomitant use with the neoplasm neovascularity inhibitor of this invention and interleukin 12 inductor shows very high anticancer activity, it is very useful as a physic constituent.

---